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NOTES

Evaluation of the INOVA Diagnostics Enzyme-Linked Immunosorbent Assay Kits for Measuring Serum Immunoglobulin G (IgG) and IgA to Deamidated Gliadin Peptides

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New assays for antibodies to deamidated gliadin peptides (DGP) expressing celiac disease-specific epitopes were evaluated using 154 sera previously tested for endomysial immunoglobulin A (IgA) (EMA), transglutaminase IgA (TGA), and conventional gliadin antibodies. DGP antibody results showed 97% concordance with EMA and TGA results. Of 56 sera negative for EMA and TGA but positive for conventional gliadin antibodies, 54 (96%) were negative for DGP antibodies.

Antibodies recognizing gliadin, the alcohol-soluble fraction of wheat gluten, are present in 80 to 90% of untreated celiac disease (CD) patients (2, 4, 12). Gliadin antibodies, however, may also be found in clinical settings other than CD, including inflammatory bowel disease, liver disease, and neurologic disorders (7, 9). Assays measuring immunoglobulin A (IgA) to endomysium and transglutaminase (the major antigenic component of endomysium) (6) exhibit better sensitivity and specificity for CD and have replaced conventional gliadin antibody assays as the best serologic tools for diagnosing CD (8, 14, 16).

Recent findings have demonstrated that gliadin-reactive antibodies from CD patients recognize a limited number of specific epitopes and that gliadin antibodies from non-CD patients rarely recognize these epitopes (1, 15). Those studies also showed that gliadin antibodies from CD patients exhibit enhanced binding to gliadin that has been deamidated by the enzymatic action of transglutaminase (1, 15). Based on this new information, INOVA Diagnostics has developed assays for IgG and IgA recognizing deamidated gliadin peptides (DGP) bearing epitopes specific for CD. These assays were evaluated in a reference laboratory setting using sera previously tested for other serologic markers of CD.

The evaluation panel contained 154 selected serum samples previously tested in a CD antibody panel that includes endomysial IgA (EMA), transglutaminase (TG) IgA (TGA), and conventional gliadin IgG and IgA. The selected sera exhibited one of three reactivity profiles in the CD antibody panel: 44 samples were positive for EMA and TGA (profile A), 56 samples were negative for EMA and TGA but positive for conventional gliadin IgG and/or IgA (profile B), and 54 were negative for all four analytes (profile C). No specimens discordant for EMA and TGA were identified during the sample collection period.

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EMA was measured by indirect immunofluorescence using monkey esophagus (Binding Site, San Diego, CA) as a substrate; sera were screened at a 1:5 dilution and titered to endpoint if positive (11, 13). Conventional gliadin IgG and IgA were measured by home-brew enzyme-linked immunosorbent assays (ELISAs) employing gliadin prepared from wheat gluten (Sigma-Aldrich, St. Louis, MO) (11, 13). TGA was measured using the INOVA Diagnostics (San Diego, CA) ELISA kit; this assay utilizes native TG purified from human erythrocytes. DGP IgG and IgA were measured using new ELISA kits supplied by INOVA Diagnostics; the sequences of the peptides used in the assays are proprietary. As with most other INOVA ELISA kit assays, these assays utilized serum diluted 1:101 and a prediluted calibrator serum enabling expression of results in units; values of <20 units were considered negative, whereas values of ≥ 20 units were considered positive.

The results are summarized in Table 1. Due to the excellent sensitivity and specificity of EMA and TGA for CD (3, 8), the 44 samples positive for EMA and TGA were presumed to represent CD patients; 40 of 44 (91%) were positive for conventional gliadin IgG and/or IgA, and 43 of 44 (98%) were positive for DGP IgG and/or IgA. The 56 samples negative for EMA and TGA but positive for conventional gliadin antibodies were presumed to represent non-CD patients; 54 of 56 samples (96%) were negative for DGP antibodies. One of the two discordant samples showed an IgG-positive–IgA-negative pattern with both conventional gliadin and DGP, suggesting the possibility of IgA-deficient CD (5, 10); however, the total IgA level (155 mg/dl, measured by nephelometry) indicated IgA sufficiency. The 54 samples negative for EMA, TGA, and conventional gliadin antibodies were also presumed to represent non-CD patients; all 54 samples (100%) were negative for DGP IgG, and 53 of 54 (98%) were negative for DGP IgA. Thus, 43 of 44 samples positive for EMA and TGA were positive for DGP antibodies, and 107 of 110 samples negative for EMA and TGA were negative for DGP antibodies, for an overall concordance rate of 97% (150/154).

TABLE 1. Results for sera used to evaluate the DGP antibody assays from INOVA Diagnostics

Profile	CD antibody panel result[a]					No. of samples exhibiting DGP antibody result pattern[b]			
	EMA	TGA	Gliadin IgG	Gliadin IgA	Total no. of samples	IgG^+ IgA^+	IgG^+ IgA^-	IgG^- IgA^+	IgG^- IgA^-
A	+	+	+	+	32	32	0	0	0
	+	+	+	−	3	2	1	0	0
	+	+	−	+	5	4	0	1	0
	+	+	−	−	4	3	0	0	1
B	−	−	+	+	10	0	0	0	10
	−	−	+	−	35	0	1	1	33
	−	−	−	+	11	0	0	0	11
C	−	−	−	−	54	0	0	1	53

[a] +, positive result; −, negative result.
[b] Results represent the number of samples exhibiting the indicated result pattern.

These findings demonstrate the very strong agreement between the detection of DGP antibodies and the detection of EMA and TGA in sera submitted for testing in a CD serologic marker panel. Because the major issue with conventional gliadin antibody detection is the lack of specificity for CD (7–9), a large proportion of the samples selected for evaluation were positive for conventional gliadin antibodies but negative for EMA and TGA; only two of these samples contained antibodies to DGP, demonstrating the superior specificity of the DGP antibody assays over conventional gliadin antibody assays. A single serum sample contained DGP IgA in the absence of the other analytes investigated; this unexpected reactivity remains unexplained and merits further investigation.

A limitation of this study is the lack of clinical information for the patients whose sera were selected for evaluation. As many studies have shown (3, 8, 13, 14, 16), however, the sensitivity, specificity, positive predictive value, and negative predictive value of EMA and TGA for CD are all well above 90%. Thus, a serum panel selected on the basis of EMA and TGA results serves as a good surrogate for a serum panel from clinically defined patients with CD.

Measurement of DGP antibodies should be a valuable adjunct to current serologic panels for diagnosing CD, particularly in those situations where EMA and/or TGA results are unclear. Furthermore, the DGP IgG assay, like the TG IgG assay, may prove useful for diagnosing CD in individuals with IgA deficiency (5, 10). Identifying this small subset of CD patients is a challenge, since sera from these individuals are falsely negative for EMA, TGA, and presumably DGP IgA. Further studies are needed to explore this hypothesis.

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